


REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 01/00/91		3. REPORT TYPE AND DATES COVERED					
4. TITLE AND SUBTITLE LABORATORY STUDIES ON BIODEGRADATION OF ORGANICS IN THE SOUTH TANK FARM PLUME (STFP) AQUIFER, SOIL AND MICROBIOLOGICAL ANALYSES OF STFP AQUIFER CORE SAMPLES				5. FUNDING NUMBERS					
6. AUTHOR(S) SALANITRO, J.; WISNIEWSKI, H.									
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) SHELL OIL COMPANY DENVER, CO				8. PERFORMING ORGANIZATION REPORT NUMBER 91100R01					
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSORING/MONITORING AGENCY REPORT NUMBER					
				NTIS CRA&I <input checked="" type="checkbox"/> DTIC TAB <input type="checkbox"/> Unannounced <input type="checkbox"/> Justification					
				By _____ Distribution /					
12a. DISTRIBUTION/AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED				12b. DISTRIBUTION CODES <table border="1"> <tr> <td>Dist</td> <td>Avail and/or Special</td> </tr> <tr> <td>A-1</td> <td></td> </tr> </table>		Dist	Avail and/or Special	A-1	
Dist	Avail and/or Special								
A-1									
13. ABSTRACT (Maximum 200 words) THIS REPORT CONTAINS A BASE SET OF DATA ON MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS OF REPRESENTATIVE SOIL CORES TAKEN FROM THE SOUTH TANK FARM PLUME (STFP). FIVE SOIL CORES WERE ANALYZED FOR VARIOUS BACTERIAL TYPES AND SOIL CHARACTERISTICS (PH, SOIL CLASS, MINERAL NUTRIENTS). THE BASIC SOIL DATA INDICATE THAT SIGNIFICANT NUMBERS OF BACTERIA AND LEVELS OF NUTRIENTS ARE PRESENT IN THE STFP GROUND WATER TO ENHANCE THE AEROBIC BIODEGRADATION OF ORGANIC COMPOUNDS IN THE AQUIFER.									
14. SUBJECT TERMS GROUNDWATER CONTAMINATION				15. NUMBER OF PAGES					
				16. PRICE CODE					
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT						

19950710 014

91100R01
2ND COPY

19950710 014

DTIC QUALITY INSPECTED 8

LABORATORY STUDIES ON BIODEGRADATION OF ORGANICS
IN THE SOUTH TANK FARM PLUME (STFP) AQUIFER

Soil and Microbiological Analyses of STFP Aquifer Core Samples

J. P. Salanitro and H. L. Wisniewski

Summary: Soil and microbial analyses were made on samples of aquifer material taken from borings within the organics plume of the South Tank Farm area (STFP). These soils (pH 7.4-8.3) were classified as loams, silty clays or clayey types and contained low levels of organic carbon (<0.1 or 0.2%) and varying concentrations (3-120 ppm) of available nutrients (Fe , $\text{NH}_4^+\text{-N}$, $\text{PO}_4^{3-}\text{-P}$). Enumeration of culturable micro-organisms associated with this aquifer material indicated that aerobic and facultatively anaerobic bacteria were present (10^3 - 10^4 /g wet soil) at all depths (19-25 ft) sampled. Anaerobic bacteria (denitrifiers, sulfate-reducers and methanogens) could not be cultured from any sample. These basic soil data indicate that significant numbers of bacteria and levels of nutrients (NH_4^+ , PO_4^{3-}) are present in the STFP groundwater to enhance the aerobic biodegradation of organic compounds in the aquifer.

Introduction

It has previously been reported that the predominant mechanism for the natural remediation of aromatic hydrocarbons in an aquifer is the stimulation of aerobic biodegradation by indigenous soil bacteria.¹⁾ Microbial populations present in aquifer material where these transformations have occurred were primarily aerobic bacteria.^{2,3)} It would be important to establish, therefore, the microbial and nutrient status of the RMA - South Tank Farm plume (STFP) aquifer to evaluate the bioremediation potential of this groundwater. The current report contains a base set of data on microbiological and chemical characteristics of representative soil cores taken from the STFP. Five soil cores were analyzed for various bacterial types and soil characteristics (pH, soil class, mineral nutrients).

Materials and Methods

Field Site Sampling. Subsurface aquifer samples were taken from five borings in the STFP representing areas in which high or low levels of aromatic hydrocarbons were present in the groundwater. Borings were made with a drilling rig and hollow stem auger (4-6 inch diameter). When desired depths were drilled, two inch X five foot cores were taken using a Waterloo saturated sand sampler with a wireline piston core barrel as described previously.⁴⁾ The stainless steel core barrel contained a two inch X five foot polybutyrate (Shelby) tube into which the soil was retained. Aquifer cores were retrieved from depths of 1-5 feet below the top of the water table. After cores were obtained they

were cut in half, capped, sealed, and sent on ice within 24-48 hours to Westhollow Research Center for processing. The field sampling was conducted by MK-Environmental Services of Denver, Colorado.

Chemical and Microbiological Soil Analyses of Aquifer Material.

Samples from each boring were sent to Soil Analytical Services, Inc. of College Station, Texas for various soil determinations such as pH, CEC (cation exchange capacity), type classification, moisture and mineral nutrients (Fe , NH_4 , PO_4^{3-}).

Microbial populations in soil were enumerated by tube extinction dilution culture methods. Endpoints of serial 1:10 dilutions of a soil inoculum made into culture media were the highest dilution showing turbid or microscopically discernible cell growth (aerobes, anaerobes), FeS precipitation from H_2S -producing sulfate-reducing bacteria, methane formation in the headspace of culture vials for methanogens or loss of NO_3^- for denitrifiers. Denitrifiers, sulfate-reducers and methanogens were estimated by culturing in denitrifying medium (0.8% Difco Nutrient Broth, 0.1% KNO_3 , and 0.5% succinate); modified Postgate medium E supplemented with 0.1% sodium acetate³⁾ and Balch medium I,⁶⁾ respectively. Total anaerobes were determined by turbidity and microscopic examination of growth in soil dilutions of modified Postgate E and Balch I media. Soil cultures were incubated at 30-35°C for 14 days.

Results and Discussion

Soil Analyses. Table 1 is a summary of physical and chemical characteristics of the soil cores taken from the STFP aquifer. These saturated soils are slightly alkaline (pH 7.4-8.0), contain low levels of organic carbon (<0.1 or 0.2%) and are classified as a loam, silty clay loam or clayey types. The CEC for the samples varied from 14-53 meq cations/100g and was similar to those reported for loams, silty clays and clayey soils.⁷⁾ The soluble Fe and Mn content varied among the five soil cores from 10-86 ppm. NH_4^+ -N was 2.5 - 9.6 ppm and available PO_4^{3-} -P concentrations were 2.5 - 117 ppm. The total phosphorus (P) content was variable (155 - 1274 ppm) between samples. The NO_3^- -N levels were less than the detectable level of 1 ppm and the organic-nitrogen (TKN) content was also low (0.07-0.2%).

Soil Microbial Populations. Soils cultured on various media for viable aerobic and anaerobic bacteria showed (Table 2) that aerobes and facultative anaerobes (microbes capable of growth under high or low dissolved oxygen conditions) were present at levels 10^3 - 10^4 /g wet soil. Similar levels of these microbes were determined from aquifer material taken where the groundwater containing high (≥ 40 ppm) or low (ppb) concentrations of aromatic hydrocarbons. These results indicate that high levels of hydrocarbon in the groundwater have not adversely affected the viability of microbes in the saturated zone. It should be noted that very similar levels of aerobic bacteria have been cultured from sandy aquifer material from Michigan and Florida.^{2,8)} Significant numbers of

other anaerobic and obligately anaerobic bacteria such as denitrifiers, sulfate-reducers and methane-formers could not be cultured from any soil core. These findings also confirm that the STFP is predominately an oxygenated aquifer containing primarily aerobic microorganisms.

References

- 1) Salanitro, J. P. and H. L. Wisniewski. Laboratory Studies on Biodegradation of Organics in South Tank Farm Plume Aquifer Samples. RMA Project Status Report 6-90.
- 2) Barker, J. F. and G. C. Patrick. 1987. Natural Attenuation of Aromatic Hydrocarbons in a Shallow Sand Aquifer. Ground Water Monitoring Rev. 7:64-71.
- 3) Chiang, C. Y., J. P. Salanitro, E. Y. Chai, J. D. Colthart and C. L. Klein. 1989. Aerobic Biodegradation of Benzene, Toluene and Xylene in a Sandy Aquifer - Data Analysis and Computer Modeling. Ground Water 27:823-834.
- 4) Zapico, M. M., S. Vales and J. A. Cherry. 1987. A Wireline Piston Core Barrel for Sampling Cohesionless Sand and Gravel below the Water Table, Ground Water Monitoring Rev. 7:74-82.
- 5) Postgate, J. R. 1979. The Sulfate Reducing Bacteria, Cambridge University Press, London.
- 6) Balch, W. E., G. E. Fox, L. J. Magnum, C. R. Woese and R. S. Wolfe. 1979. Methanogens: Reevaluation of a Unique Biological Group. Microbiol. Rev. 43:260-296.
- 7) Lyon, T. L., H. O. Buckman and N. C. Grady. 1952. The Nature and Properties of Soils, 5th ed. New York:Macmillan.
- 8) Kemblowski, M. W., J. P. Salanitro, G. M. Deeley and C. C. Stanley. 1987. Fate and Transport of Residual Hydrocarbons in Ground Water - A Case Study. In Proc. Petrol. Hydrocarbons and Organic Chemicals in Ground Water, Nat. Well Water Association/Amer. Petrol. Inst. Conf., November.

Table 1

Physical and Chemical Soil Analyses on Aquifer Core
Material from the STFP

<u>Parameter*</u>	<u>Core Sample (depth, ft)</u>				
	<u>01552-B (19-21)</u>	<u>01588-B (19-21)</u>	<u>01588-B (21-23)</u>	<u>02506-B (23-25)</u>	<u>02579-B (17-19)</u>
pH	7.8	7.9	7.7	7.4	8.3
% Moisture	18	28	29	29	23
CEC, meq/100g	24	53	52	48	14
Texture % Sand/Silt/Clay	13/53/33	42/30/27	44/30/26	43/33/23	39/18/43
Soil Class	silty clay loam	loam	loam	loam	clay
Organic Carbon, %	<0.1	<0.1	<0.1	<0.1	<0.2
Minerals, ppm					
Fe	19	62	86	50	10
Mn	29	53	31	61	22
NH ₄ ⁺ -N	9.6	6.5	8.3	2.5	5.3
PO ₄ ⁻³ -P	2.4	79	117	62	42
Total P	177	155	1274	1146	371

* In all soil samples NO₃-N was <1 ppm and the TKN varied 0.07-0.2%.

Table 2
Enumeration of Microbial Populations
in Aquifer Core Material from the SFP

<u>Core Sample (ft)^{a)}</u>	<u>No. of Bacteria/g Wet Soil</u>		
	<u>Aerobes</u>	<u>Facultative Anaerobes^{b)}</u>	<u>Hydrocarbons in Groundwater^{c)}</u>
1) 01552-B (19-21)	10 ⁴	10 ³	High
2) 01588-B (19-21)	10 ³	10 ³	High
3) 01588-B (21-23)	10 ⁴	10 ³ -10 ⁴	High
4) 02506-B (23-25)	10 ⁷ -10 ⁴	10 ³ -10 ⁴	Low
5) 02579-B (17-19)	10 ⁴	10 ³ -10 ⁴	Low

a) Depth below ground level. Cores were taken 1-5 ft below the top of the water table.

b) Other anaerobic bacteria (denitrifiers, sulfate-reducers and methane-forming organisms) could not be cultured from any sample (less than the detection level of 10²/g).

c) Total aromatic hydrocarbons in ground water samples were ≥ 40 ppm (high) or ppb levels (low).